MICROBIAL CELLULOSE PRODUCTION BY *ACETOBACTER XYLINUM* AND ITS APPLICATION FOR THE FASHION AND TEXTILE INDUSTRY

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Abstract: *This paper will give an overview on the bioprocess for microbial cellulose material production and its application for the fashion and textile industry. The process utilizes Acetobacter xylinum, a non-hazardous and non-pathogenic bacterium, to produce cellulosic nano-fibers -chemically similar to cotton-. These nano-fibers form a dense structure, as we call it, the Microbial Non-Woven (MNW). Compared to cotton, MNW possesses superior properties such as high purity and density, shape retention, high water uptake, enhanced tensile strength, and larger surface area when wet. Our research aims to also maintain these superior properties when the MNW is dried. In addition, MNW production requires less water for irrigation and harmful substances, both for growing and for processing, which makes the material more environmentally friendly and sustainable. The characteristics of MNW with respect to its structure and physicochemical properties are discussed as well as current and potential applications in food, medicine, electronics, pharmaceutical, textile and other industries.*

Keywords: *Microbial cellulose, non-woven, Acetobacter xylinum, bioprocess*

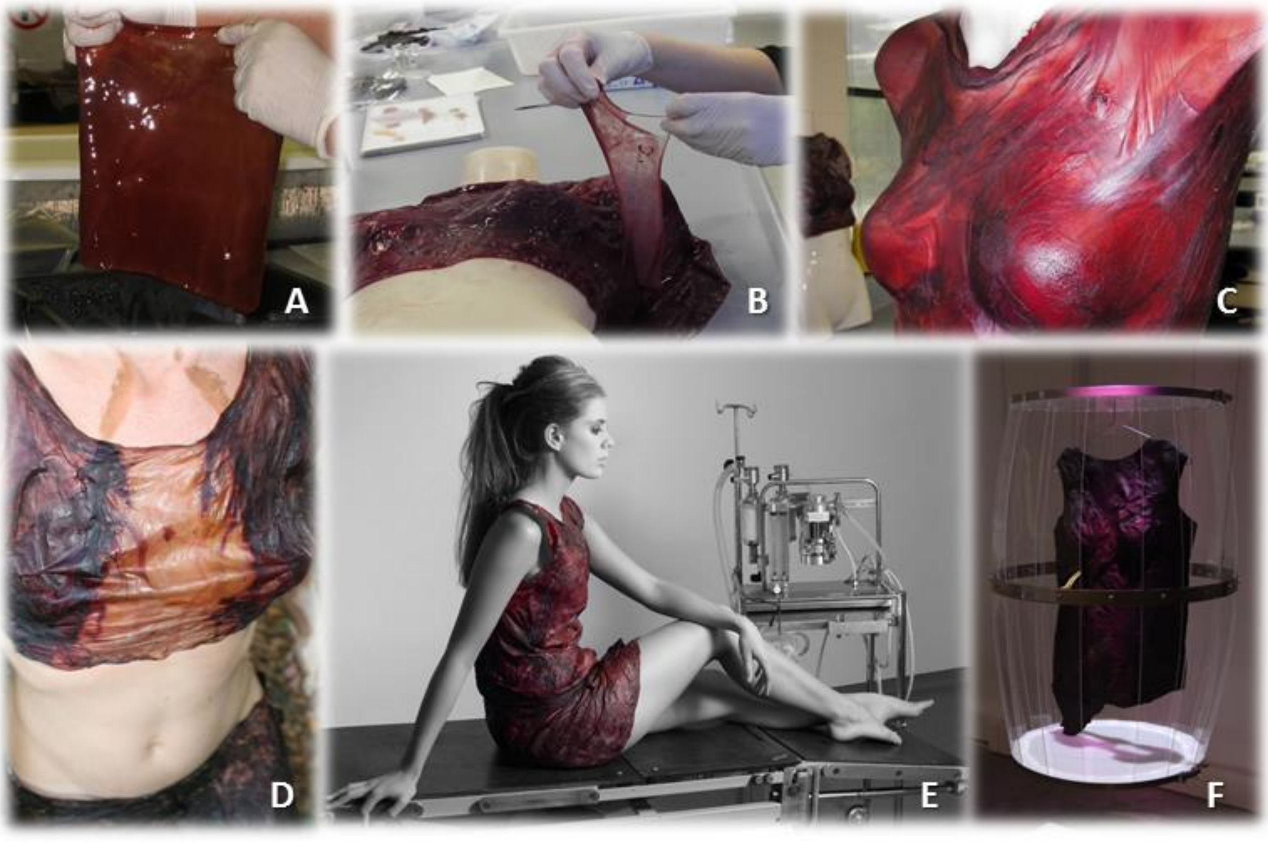

Figure 1: Creating a fashion item from wine fermentation. **A**: harvesting microbial cellulose from culture media, **B** and **C**: casting the material onto scaffold and drying, **D**: a variety of colors is achievable controlled by the nutrition, **E** and **F**: the original wine dress made in 2007, from the Cité des Science in Paris and CETI, Centre Européens des Textiles Innovants, Lille. Both 2013

1. Introduction

A. xylinum is known as a bacterium causes wine spoilage, transforming wine into vinegar. The by-product of this activity is the formation of cellulose, a slimy, rubbery, soft, skin-like substance. This substance termed the ‘vinegar mother’ originates from the ancient European; a few millenniums ago they believed that it was the skin of mother that ‘gives birth’ to the vinegar once it was placed in the wine. *A. xylinum* are rod shaped bacteria, 1-4μm in size, aerobic (consuming oxygen) and can be found to be motile or non-motile. *A. xylinum*'s ability to synthesize large quantities of micro fibrils of pure cellulose can possibly be explained by [1] who terms the material a biofilm; a type of cellulitic raft that floats the bacteria close to the surface of the liquid where the most oxygen is found. From an ecological view point, the Acetobacter has evolved to deal with the high alcohol and acidic environments of decaying plant material left behind by yeasts and other micro-flora [2].

It has been known for more than a century that some bacteria such as *Acetobacter, Agrobacterium, Achromobacter, Aerobacter, Sarcina, Azotobacter, Rhizobium, Pseudomonas, Salmonella* and *Alcaligenes* can biosynthesis cellulose in the form of interwoven extracellular ribbons as part of their primary metabolite [3, 4]. Production of cellulose from *A. xylinum* was first reported in 1886 by A.J. Brown [5] in Europe. He observed that the resting cells of Acetobacter produced cellulose in the presence of oxygen and glucose. The gram-negative bacterium, *A. xylinum*, has been applied since then as the model microorganism for basic and applied studies due to the fact that it can polymerize up to 200,000 glucose molecules per second [6,7] from a wide range of carbon and nitrogen sources into β-1,4-glucan chains, forming ribbon-like bundles of micro-fibrils.

The resulting cellulosic micro-fibril material, the MNW, possesses several unique properties such as high purity and density, shape retention, high water binding capacity, enhanced tensile strength, and large surface area. These complementary features make the MNW a suitable candidate for a wide variety and diverse potential for future applications. Furthermore, the MNW material is of much interest to the fashion and textile industry compared to the conventional cellulose sources such as the cotton plant, *Gossypium*. However, at the current state, the MNW can not be used as a textile material as its physical properties are strong functions of the amount of humidity it hosts.

Therefore, our research is based on addressing two main issues;

- one being the optimisation of multiple parameters such as strain, growth medium, environmental conditions and formation of by-products in order to obtain the optimum material commercially attractive and industrially possible
- the other being applying as well as optimising a specific cross-linking chemistry to suppress heavy dependence of its physical properties on humidity which would potentially turn MNW into a textile fabric suitable for garment making

both for allowing large scale production in textile industry.

This review offers a brief introduction to some of the guiding concepts currently adopted in the investigation of microbial cellulose biosynthesis and a synopsis of the many recent developments reported for the microbial cellulose production and its application as-grown. In addition to its bio-synthesis processes, an outline on bio-reactors and cross-linker chemistry will also be briefly discussed.

2. Material and Methods

We will outline some of the experimental techniques that are currently employed as well as novel ones to be applied for both the pre- and post-culture production of the MNW material for industrial scale. The material and garments of Fig. 1 was made from the bacterial fermentation of wine, beer or most alcoholic beverage as a possible pathway is seen in Fig. 2. Culture of naturally occurring *A. xylinum* was employed, a vinegar producing bacteria, in a vat of wine. The *A. xylinum* have the central role in this process by converting the alcohol as well as any suitable carbon source in the vat into cellulosic micro-fibres. This microbial cellulose is chemically similar to cotton. Therefore, the garments are made from microbial cotton. Depending on the size or shape of the garment, we used different size vats. The microbial cotton was formed on the surface of the wine, almost as if the bacteria are trying to form a raft to flow on the wine in order to have access to oxygen in the air. The bacteria's end product is vinegar, which makes the garment smell a bit pungent; however, the garments are more environmentally friendly than genetically engineered cotton plants. The 2-dimensional form that we extracted from the vat is then formed into a garment.

We have now perfected a culturing technique that will allow the bacteria to form a 3-dimensional garment that will be seamless. It can also be formed to fit the wearer like a second skin.

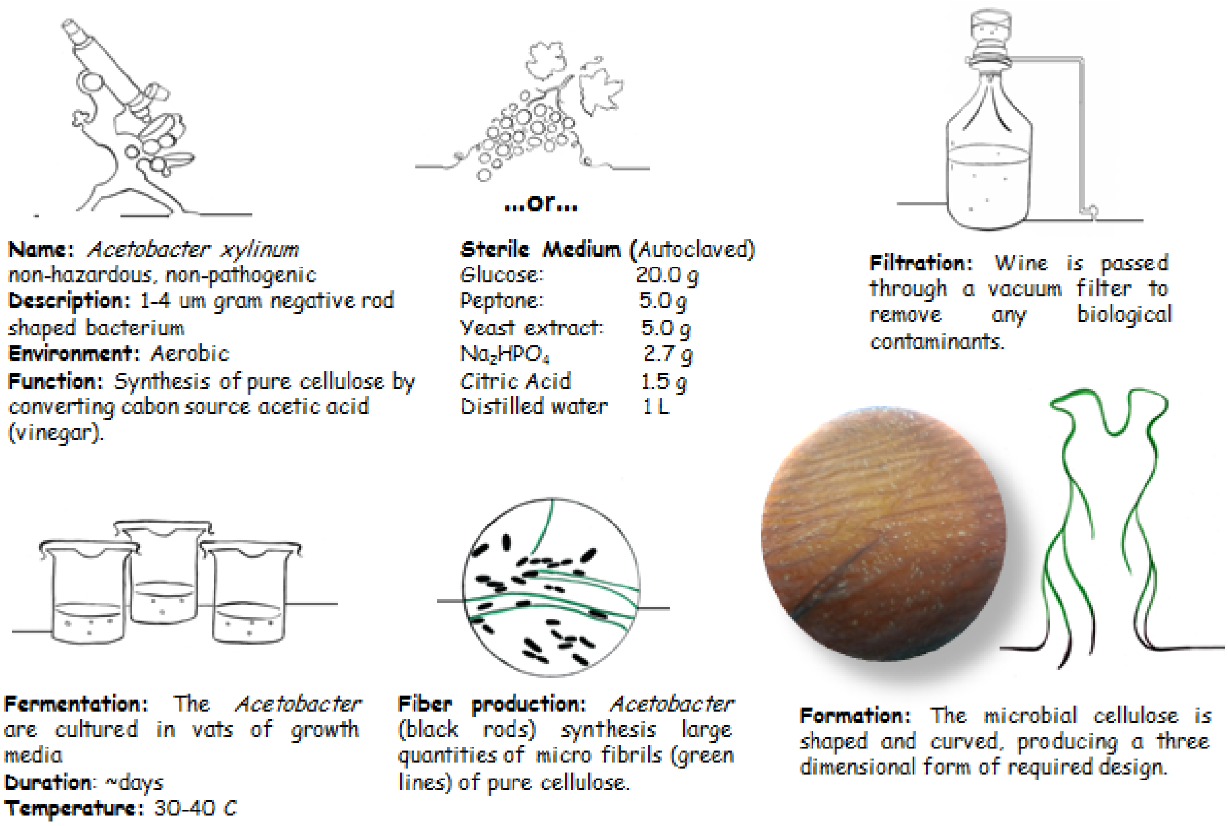

Figure 2: A possible pathway for fermented fashion

However, this natural straight-forward way of fermentation can be accelerated by a few orders of magnitude via optimization of the fermentation media by means of strain selection and conditioning, choice of nutrition, bio-reactor design, and applied environmental stress onto the culture such as pressure, external electro-magnetic fields, etc.

2.1 Strain Selection

It is known that different strains have different efficiencies to biosynthesise cellulose even when grown under the same conditions. For example two strains of *A. xylinum*, ATCC23769 and ATCC53582, have been studied for the production of cellulose. Although, genetically highly similar, the ATCC53582 strain produces 5 times the amount of cellulose compared to ATCC23769 during a 7-day incubation, even though, the cell growth of ATCC23769 was twice that of ATCC53582 [8]. Therefore, a large scale screening of naturally occurring sources for cellulose producing strains and isolation of high rate production strains is essential.

2.2 Biosynthesis pathways

Even though, converting carbon sources such as alcohol and sugars into MNW is an enzymatic process, which takes place within the bacterial cell wall, the biological pathway is not uniquely defined. There are multiple possible pathways with varying efficiency, yield, quality, and environmental requirements where synergistic effects might be searched for by means of design of experiment approaches.

Enhancement of the microbial conversion of the carbon sources into bacterial cellulose via biosynthesis includes the following steps:

- Production of Acetobacter mutants by applying external means to the growth culture media such as UV irradiation, high hydrostatic pressure, EM field, chemical exposures, etc.
- *In-situ* controlling the pH, temperature, humidity, oxygen and nitrogen levels via an optimized fermentation medium design,
- Variation of carbon sources such as mannitol, fructose, xylose, sucrose, arabitol, glycerol or mixture of those,
- Promotion of the bacterial growth and MNW production by adding supplements or additional substrates to the medium such as lactate, ethanol, chitosan and others,

2.3 Mutagenesis for strain improvement

In the recent years, several studies described mutagenesis of a variety *A. xylinum* strains by means of UV irradiation, chemical exposures, high hydrostatic pressure or genetic mutations to improve the microbial cellulose yield. Enhanced cellulose production was successfully performed on *A. xylinum* strain ATCC 10245

by applying ultraviolet (UV) irradiation and/or N-methyl-N'-nitro-N-nitrosoguanidine (NTG) mutagens [9]. Single mutagen treatment improved the MNW yield significantly (~50%) while sustaining genetically stable strain that do not revert to the parent. However, the combined treatment of chemical and physical mutagens did not produced synergistic effects and did not improve MNW yield compared to that of a single mutagen. Additionally, the effects of the following external stimuli exerted onto the culture are of great interest today:

- High hydrostatic pressure which alters temporarily the behaviour of the strains without genetic modification [10]
- Mild sonication and altered gravitation
- External electro-magnetic fields, light with various frequencies, etc.

2.4 Bioreactor

Once the final decision on the strain to be utilized is made, then the incubator system as the environment in which the bio-synthesis of MNW will take place becomes an issue. The bio-reactor should in principle be as efficient as possible which involves many conflicting parameters to be optimized concurrently, such as mechanical shaking:

- culture media should be shaken with a certain frequency and amplitude to increase the possibility for the bacteria to meet the nutrition, which in turn would lead to undesired aggregated cellulose synthesis, and
- the culture media should be as stable as possible for the final MNW material to have the desired fibrous structures instead.

There are mainly the following three reactor types and their custom hybrids reported in the literature:

- *Rotating disk reactor*, where a collecting plate is placed partly inside the growth medium, picking up synthesized cellulosic structures from within the solution continuously
- *Static reactor*, where the growth medium is stable and the synthesized cellulose is harvested from the top of the solution at the air-medium interface
- *Aerosol reactor* where the nutrition is sprayed onto the air-medium interface from the air side in order to avoid the wall effect for higher yield

2.5 Applications As-Grown

MNW as-grown, both alone or within a composite structure, finds a diverse area of application from food thickeners to aircraft bodies. The applications are not even limited to the followings [11, 12]: In *food industry*, MNW is used in desserts, as thickeners in ice-cream and salad dressings, as weight reduction bases or as base for artificial meat, as sausage and meat casings, serum cholesterol reduction, etc. In *healthcare and cosmetics*, MNW is used as wound dressings and drug delivery media -either oral or dermal-, artificial skin substrate, skin creams, astringents, base for artificial nails. In *environmental sciences*, MNW finds its applications as absorptive base for toxic material removal, mineral and oil recovery, water purification as reverse osmosis membrane, disposable and recyclable diapers, etc. In *forestry*, MNW is utilized in artificial wood strengthener such as those in plywood laminates, as filler for paper, and in high strength containers, etc. Special paper industry uses commonly in archival document repair, as paper base for long-lived banknotes, as superior audio speaker diaphragms, in artificial leather products, in disposable tents and in camping gear. In *avionics* and in *automotive industries*, MNW is used within composites for car and airplane structural elements, and rocket casings for deep space missions, etc.

Considering *textile and fashion industry*, MNW does not find a broad range of applications due to the fact that its physical properties strongly depend on the humidity it hosts, giving rise to a wide range of physical forms, preventing it from being used as a textile fabric. Therefore, the research on MNW as a textile fabric should focus on suppressing this dependency by chemical treatment such as cross-linking.

2.6 Cross-Linking Chemistry

Having a high degree of polymerization (DPn) does not only lead to physically well entangled fibres within a non-woven matrix but also provides high elastic modules up to GPa orders of magnitude, surprisingly comparable to and occasionally higher then steel. This makes MNW material a promising candidate for reinforcement element within composites which could be prepared both *in-situ* such as concurrent complex formation and *ex-situ* such as post-functionalization.

One of the commonly used techniques for *in-situ* modification of MNW is via adding to the medium a variety of substances like polymers such as polyvinylalcohol, chitosan, and chitin [13] and as complex-forming metal salts, which are capable of binding to the cellulose micro-fibrils currently being synthesized by the bacteria.

Another way to access MNW composites is an *ex-situ* preparation via impregnation of desired substances such as monomers into the thin film of MNW, followed by curing [14, 15]. A variety of curing methods are available for MNW-based composites such as thermal, microwave or UV. The features of the impregnated

monomers are also expected to appear in the final composite. For instance, metal nano-particle/MNW composites can be prepared via *in-situ* reduction of impregnated metal salts onto the cellulosic nano-fibre surface [16], which gives the final composite different colours and other desired features such as antibacterial activity, and UV absorbance characteristics.

3. Conclusion

The idea of bio-synthesizing full garment pieces out of substances added into a solution and a straight-forward chemical cross-linking treatment after can potentially obviate majority of the steps involved in standard garment production. These involve but not limited to spinning, weaving, cutting, sewing, and many other steps known in textile industry as pre-treatment and as finishes. The former is due to direct bio-synthesis into any shape, whereas the latter is due to its purity or to the simplicity of its surface chemistry. MNW has a double-fold beneficial impact on the environment as summarised below:

- Biosynthesis of MNW material avoids the usage of harmful substances that are necessary for *growing* natural cotton. The bio-synthesis of MNW material employs a natural process without any need for pesticide usage, which is harmful to human and to the environment due to the alteration of the local eco-system.

- Biosynthesis of MNW material minimizes the usage of harmful substances that are necessary for *processing* natural cotton. Removing *hemicellulose*, *lignin*, *wax* etc. from the cellulose itself is a concern in textile industry as it requires pre-treatment applications to break the structure, which can in turn generate toxic substances. In some pre-treatments, applying high temperature and pressure is also necessary and therefore, the energy consumption of the process is usually high.

The two issues preventing the MNW from being used widely for textile production are identified as i) the construction of industrial-scale bio-reactors and as issue of ii) suppressing the dependence of its physical properties on the amount of humidity it hosts possibly by means of a specialized cross-linking chemistry application.

Once the aforementioned issues are addressed, the MNW material could potentially change the current state-of-the-art cotton agriculture and/or even replace it. The MNW material might as well possibly change the way we produce and wear textile fabric dramatically.

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